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Local and Systemic Therapy of Human Prostate Adenocarcinoma with the Conditionally Replicating Herpes Simplex Virus Vector G207

JONATHAN R. WALKER,^{1,2} KEVIN G. McGEACH,^{1,2} PERIASAMY SUNDARESAN,^{1,3}
TIMOTHY J. JORGENSEN,^{4,5} SAMUEL D. RABKIN,^{1,3,6} and ROBERT L. MARTUZA^{1,3}

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ABSTRACT

Prostate adenocarcinoma is the most common nonskin malignancy in males and the second most common cause of cancer death in the United States (Landis *et al.*, 1998). Initial treatments of surgery or radiotherapy may cause impotence and/or incontinence from neural damage (Eastham and Scardino, 1993; Porter *et al.*, 1998). When extraprostatic or metastatic disease develops, castration or pharmaceutical androgen ablation is utilized (Catalona, 1994). Androgen-resistant recurrence indicates a poor prognosis and justifies experimental chemotherapy (Oh and Kantoff, 1998). G207 (Mineta *et al.*, 1995; Yazaki *et al.*, 1995) is a multimitated herpes simplex virus 1 (HSV) vector that replicates within cancer cells, causing cellular death; however, replication is limited in normal cells, including those of the nervous system. *In vitro*, G207 at a low multiplicity of infection (MOI of 0.01) is oncolytic for multiple human prostate cancer cells. In athymic mice, a single intraneoplastic inoculation of G207 completely eradicates >22% of established subcutaneous human prostate cancer tumors irrespective of hormonal responsiveness. Two intraneoplastic inoculations of G207 completely eradicated two of three recurrent previously irradiated tumors and two intravenous administration of G207 induced tumor regression in distant subcutaneous tumors and completely eradicated one-fourth of the tumors.

OVERVIEW SUMMARY

G207 is a multimitated herpes simplex virus 1 vector that lacks both copies of the ICP34.5 gene and contains an insertion of the *lacZ* gene inactivating the ICP6 gene. G207 can replicate within cancer cells, causing cellular death; however, replication is limited in normal cells, including those of the nervous system. *In vitro*, at a low multiplicity of infection (MOI of 0.01), G207 is oncolytic for multiple human prostate cancer cell lines (LNCaP, DU-145, PC-3, and TSUPR-1). In athymic mice, a single intraneoplastic injection of G207 (2×10^7 PFU) into established subcutaneous human prostate cancer tumors caused a reduction in tumor volume (LNCaP, $p < 0.05$; DU-145, $p < 0.01$ versus controls) with complete eradication of >22% of tumors irrespective of hormonal responsiveness (LNCaP, 25%; DU-145, 22%). Two intraneoplastic injections of G207 (1×10^7

PFU) caused tumor volume reduction ($p < 0.05$ versus controls) and completely eradicated 66% of recurrent previously irradiated LNCaP tumors. Two intravenous doses of G207 (2×10^7 PFU) induced tumor regression in distant subcutaneous tumors (LNCaP, $p < 0.001$; DU-145, $p < 0.05$ versus controls) and completely eradicated one-fourth (LNCaP, 14%; and DU-145, 40%) of the tumors. G207 and related conditionally replicating HSV vectors warrant further evaluation for the treatment of local and metastatic prostate cancer.

INTRODUCTION

VARIOUS VIRAL VECTORS have been utilized for antiprostata cancer therapy (Eastham *et al.*, 1996; Rodriguez *et al.*, 1997; Hall *et al.*, 1998). However, delivery of viral particles to

¹Molecular Neurosurgery Laboratory, ²Department of Surgery (Division of Urology), ³Department of Neurosurgery, ⁴Department of Radiation Medicine, ⁵Department of Biochemistry and Molecular Biology, and ⁶Department of Microbiology and Immunology, Georgetown University Medical Center, Washington, D.C. 20007.

every cancer cell is a major limitation of this form of experimental therapy. Replication-competent vectors offer a potential advantage over replication-defective vectors owing to the possibility of viral multiplication within the tumor after viral delivery. The genetic modifications in G207 (deletion of both ICP34.5 genes and a *lacZ* insertion inactivating the ICP6 gene) permit replication within cancer cells but limit replication in normal cells, including those of the nervous system (Mineta *et al.*, 1995; Yazaki *et al.*, 1995). In athymic mouse models in which G207 has been studied, the antitumoral effect of G207 is primarily due to virally induced lysis of the infected cancer cells (Yazaki *et al.*, 1995; Toda *et al.*, 1998a). In immune-competent animals an additional benefit is due to the induction of an immune response to the tumor (Toda *et al.*, 1998b, 1999). For example, studies have shown tumor regression of noninoculated established subcutaneous tumors in syngeneic bilateral tumor models after intraneoplastic injection of G207 into a distant tumor. This effect was not due to systemic viral spread, but to the induction of tumor-specific cytotoxic lymphocytes (Toda *et al.*, 1999). This effect was lost when similar studies were done on immune-deficient animals.

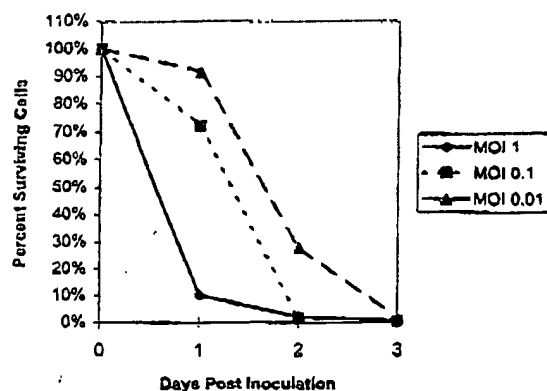
G207 was originally developed to treat malignant brain tumors without harming surrounding normal brain cells (Mineta *et al.*, 1995; Yazaki *et al.*, 1995) and is now in a phase I clinical trial for recurrent malignant gliomas. More recently, G207 has been evaluated for the treatment of nonnervous system tumors (Toda *et al.*, 1998a, 1999). Because G207 is nonneurovirulent and neural damage is the major morbidity associated with the treatment of prostate cancer, G207 provides an alternative therapeutic vector for prostate cancer.

MATERIALS AND METHODS

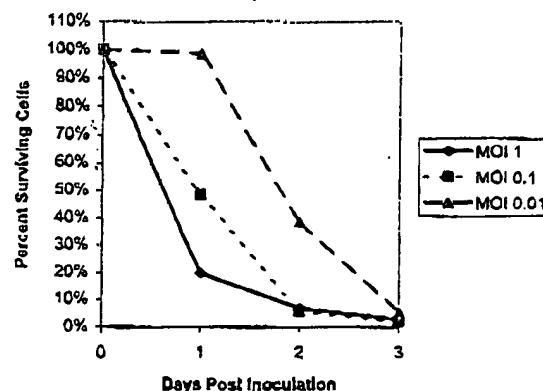
Cell lines and in vitro susceptibility testing

Human-derived prostate cancer cell lines included LNCaP (provided by E.P. Gelmann, Georgetown University Medical Center, Washington, D.C.), DU-145 and PC-3 (provided by the Lombardi Cancer Center, Georgetown University Medical Center), and TSUPR-1 (provided by W. Isaacs, James Buchanan Brady Urological Institute, Baltimore, MD). LNCaP was main-

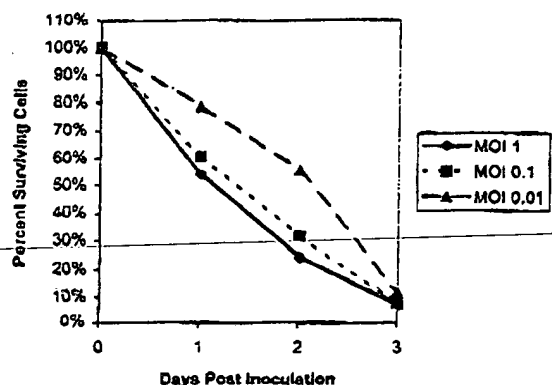
LNCaP



DU145



PC-3



TSUPR-1

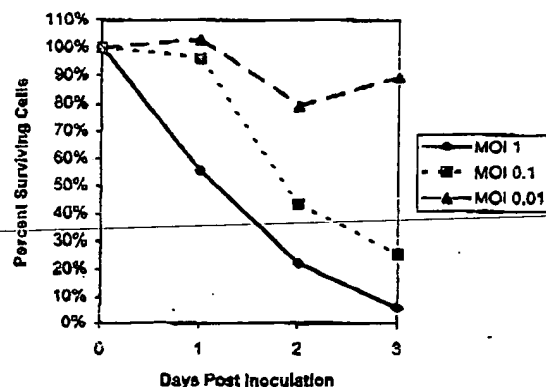


FIG. 1. G207 susceptibility of human-derived prostate cancer cell lines *in vitro*. Four human-derived prostate cancer cell lines (LNCaP, DU-145, PC-3, and TSUPR-1) were infected with G207 at MOIs of 1, 0.1, and 0.01. Each data point (mean of triplicate wells) is the percentage of surviving cells compared with the number of cells in control wells at each time point.

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tained in Iscove's modified Eagle's medium (IMEM; Biofluids, Rockville, MD) containing 5% calf serum (HyClone, Logan, UT). DU-145 and PC-3 were maintained in RPMI 1640 (Biofluids) containing 10% fetal calf serum (HyClone), and TSUPR-1 was maintained in Dulbecco's modified Eagle's medium (Biofluids) with 10% fetal calf serum. All cell lines were maintained at 37°C in 5% CO₂ with penicillin and streptomycin (Sigma, St. Louis, MO) added to the medium, and were free of mycoplasma contamination. G207 (obtained from NeuroVir, Vancouver, BC, Canada) was titrated on African green monkey (Vero) cells (provided by D. Knipe, Harvard Medical School, Boston, MA). For *in vitro* susceptibility assays LNCaP, DU-145, PC-3, or TSUPR-1 cells were plated in six-well dishes, infected with G207 at different multiplicities of infection (MOIs), and maintained in 1% heat-inactivated fetal calf serum in buffered saline at 34.5°C. Cell counts were done in triplicate using a ZM Coulter counter (Coulter, Miami, FL) for each MOI on days 1, 2, and 3 postinfection, after washing with phosphate-buffered saline (PBS) and detaching cells with trypsin-EDTA (GIBCO-BRL, Grand Island, NY).

Subcutaneous tumor model and intraneoplastic inoculation of subcutaneous tumors

Six- to 7-week-old male BALB/c *nu/nu* mice were obtained from the National Cancer Institute (Rockville, MD). The Georgetown University Animal Care and Use Committee approved all animal procedures. The mice were anesthetized with an intraperitoneal injection of a 0.25- to 0.30-ml solution consisting of 84% bacteriostatic saline (Abbott Laboratories, Chicago, IL), 10% sodium pentobarbital (1 mg/ml; Abbott Laboratories), and 6% ethyl alcohol or inhalation of 2-3 minimal alveolar concentration of methoxyflurane (Schering-Plough Animal Health, Union, NJ). Subcutaneous tumors were induced by flank injection of 5×10^6 LNCaP cells in 0.1 ml with an

equal volume of Matrigel (Collaborative Biochemical Products, Bedford, MA), or 5×10^6 DU-145 cells in 0.1 ml. Tumors were measured by external caliper to within 0.1 mm, and volumes were calculated ($V = H \times L \times W$) and recorded. Animals with tumors less than 25 mm³ were excluded. Tumors were inoculated intraneoplastically with G207 (2×10^7 PFU in 20 μ l) or virus buffer (20 μ l). Animals were sacrificed when the tumor diameter was greater than 18 mm.

Intraneoplastic inoculation in irradiated subcutaneous tumors

To study irradiated tumors, LNCaP tumors were established subcutaneously as described above, and animals randomized. Tumors received a total of 10, 20, 30, or 40 Gy fractionated over a 5-day period, using a cesium-137 irradiator or mock non-irradiated treatment. Tumors in the 10- and 20-Gy group recurred after initial radiation treatment. Animals with recurrent tumors were randomized into two new groups and the tumors were either inoculated intraneoplastically with G207 (1×10^7 PFU in 20 μ l) or virus buffer (20 μ l); repeat inoculation was done 2 days later.

Intravenous inoculation of G207

LNCaP or DU-145 tumors were established subcutaneously as described above, and randomized into two groups respectively. Animals were inoculated by tail vein with either G207 (2×10^7 PFU in 100 μ l) or virus buffer (100 μ l). This treatment was repeated 4 days later.

Serum PSA determination

At the completion of subcutaneous LNCaP tumor treatment, animals treated with either G207 (2×10^7 PFU) or virus buffer were sacrificed. Blood was obtained and centrifuged, and the

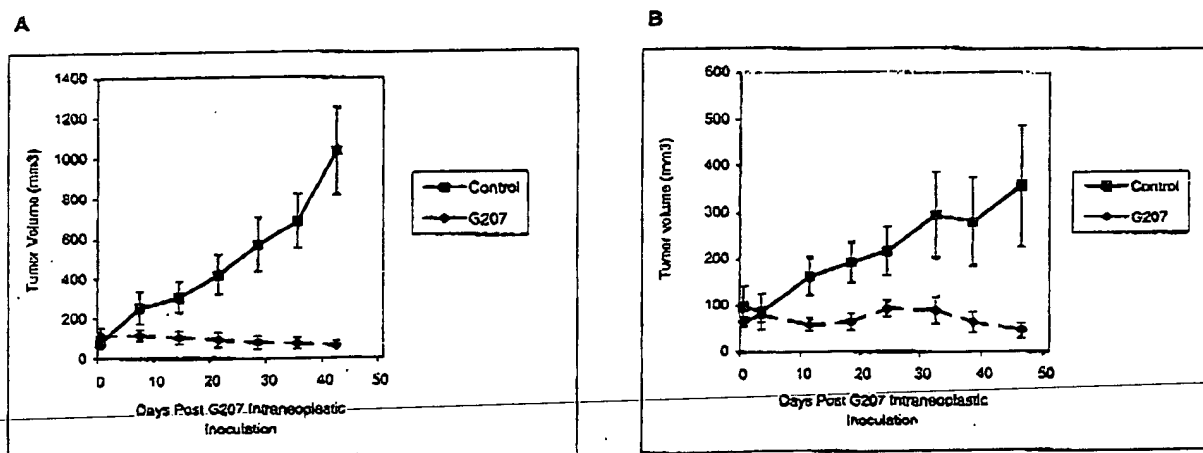


FIG. 2. The effect of intraneoplastic injection of G207 on subcutaneous human prostate tumor growth. (A) LNCaP and (B) DU-145 subcutaneous tumors were established in BALB/c *nu/nu* mice. When the mean volume of LNCaP tumors ($n = 8$) and DU-145 tumors ($n = 16$) was 99 mm³ (range, 28–224 mm³) and 80 mm³ (range, 26–350 mm³), respectively, tumors received a single intraneoplastic injection of G207 (2×10^7 PFU) or virus buffer (day 0). G207-treated tumors showed a reduction in volume, whereas buffer-treated tumors continued to grow (LNCaP, $p < 0.05$ versus controls on day 42; DU-145, $p < 0.01$ versus controls on day 46). Complete eradication of 25 and 22% of the LNCaP and DU-145 tumors, respectively, was noted. (Bars represent mean tumor volume \pm the standard error of the mean.)

serum was collected. Serum was sent to Bayer (Tarrytown, NY) for determination of total prostate-specific antigen (PSA). (PSA testing was kindly done by G. Allard.)

RESULTS AND DISCUSSION

The sensitivity of four human-derived prostate cancer cell lines (LNCaP, DU-145, PC-3, and TSUPR-1) to G207 was evaluated. Effective cytotoxicity, with >90% cell destruction within 3 days, was noted for three of four cell lines (LNCaP, DU-145, and PC-3) at an MOI of 0.01 (Fig. 1). This contrasts with studies of other viral vectors that require MOIs > 1 to achieve the same level of oncolysis for prostate cancer cell lines (Eastham *et al.*, 1996; Gotoh *et al.*, 1997). It also represents a higher level of oncolysis at lower MOIs than indicated in prior studies by us using G207 against human nervous system tumor cell lines (Yazaki *et al.*, 1995).

To study the effect of intraneoplastic G207 treatment on localized tumors, LNCaP and DU-145 were chosen for *in vivo* study since both cell lines had comparable *in vitro* oncolysis, while LNCaP is hormonally responsive and DU-145 is hormonally unresponsive (Tilley *et al.*, 1990). Hormonally unresponsive tumors are generally more aggressive, with an increased rate of metastasis and poorer patient survival (Stephenson *et al.*, 1992; Rembrink *et al.*, 1997; Oh and Kantoff, 1998). Established subcutaneous LNCaP ($n = 8$) or

DU-145 ($n = 16$) tumors in athymic mice were inoculated with either G207 (2×10^7 PFU) or virus buffer intraneoplastically. Control tumor volumes increased to the point that the animals had to be sacrificed. In contrast to the controls, G207 induced significant tumor regression of LNCaP ($p < 0.05$ versus controls on day 42, *t* test) and DU-145 tumors ($p < 0.001$ versus controls on day 46, *t* test) (Fig. 2A and B). Treatment with one inoculation of G207 (2×10^7 PFU) resulted in eradication of 25% of the LNCaP tumors and 22% of the DU-145 tumors. LNCaP tumors produce human PSA, which can be measured in the serum. Mean total serum PSA was significantly lower (0.18 ng/ml) in the G207-treated group than in the virus buffer-treated group (102 ng/ml) ($p < 0.005$ versus controls, *t* test).

Because patients who choose radiation therapy as their primary treatment may develop a localized recurrence of prostate cancer the effectiveness of G207 on previously irradiated localized tumors was studied. Subcutaneous LNCaP ($n = 15$) tumors in athymic mice were initially irradiated. All tumors not irradiated (0 Gy) progressed until all animals had to be sacrificed. All tumors treated with 40 Gy and 33% of tumors treated with 30 Gy showed complete tumor eradication without recurrence. In contrast, all tumors treated with 10 or 20 Gy showed transient tumor volume reduction followed by regrowth. These 10- and 20-Gy radiation failures were then grouped together and randomized to receive either intraneoplastic injection of G207 (1×10^7 PFU) or virus buffer with treatment repeated 2 days later. All buffer-treated tumors continued to grow, whereas

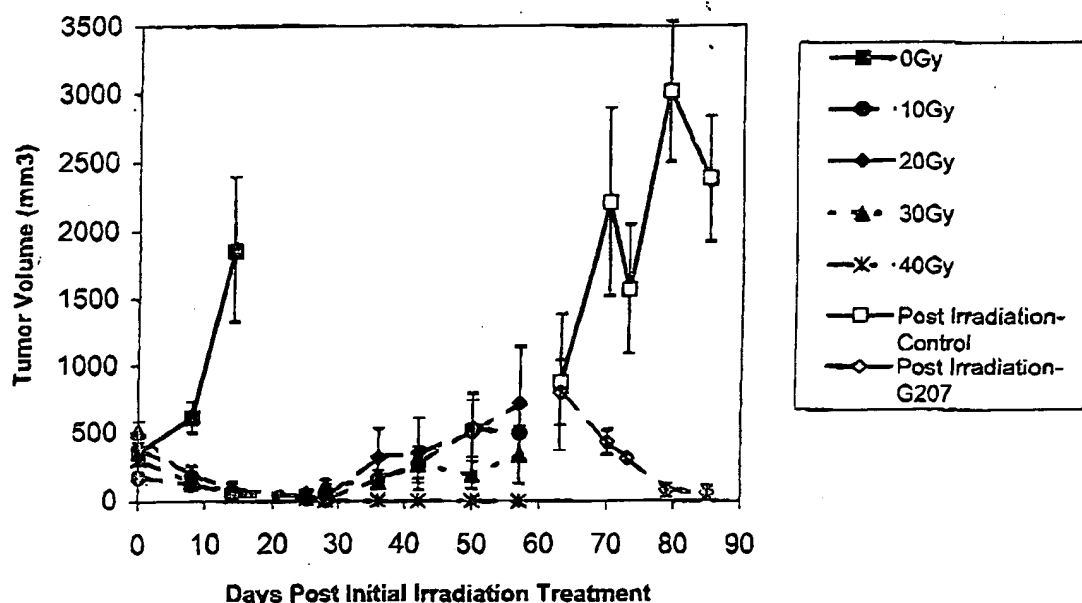


FIG. 3. The effect of radiation and G207 on subcutaneous LNCaP tumor growth. Subcutaneous LNCaP tumors were established in BALB/c *nu/nu* mice. When the mean tumor volume for LNCaP tumors ($n = 15$) was 348 mm^3 (range, $120\text{--}693 \text{ mm}^3$), tumors were randomized into five groups. Radiation was fractionated in equal doses over 5 days for a total dose of 10, 20, 30, or 40 Gy. Radiation treatment was noted to cause an initial reduction in tumor volume at all treatment doses when compared with 0 Gy. Tumor eradication was noted in 100% of the 40 Gy-treated tumors, and in 33% of the 30 Gy-treated tumors. All of these animals were then excluded from further studies. After the initial reduction in volume, all tumors in the 10- and 20-Gy treatment groups showed an increase in tumor volume. These tumors ($n = 6$) were then randomized to receive either G207 (1×10^7 PFU) intraneoplastically, followed 2 days later by a second injection, or two injections of virus buffer. G207-treated tumors had a marked reduction in tumor volume ($p < 0.05$ versus control on day 86) and tumor eradication was noted in 60% of the G207-treated tumors. (Bars represent mean tumor volume \pm the standard error of the mean.)

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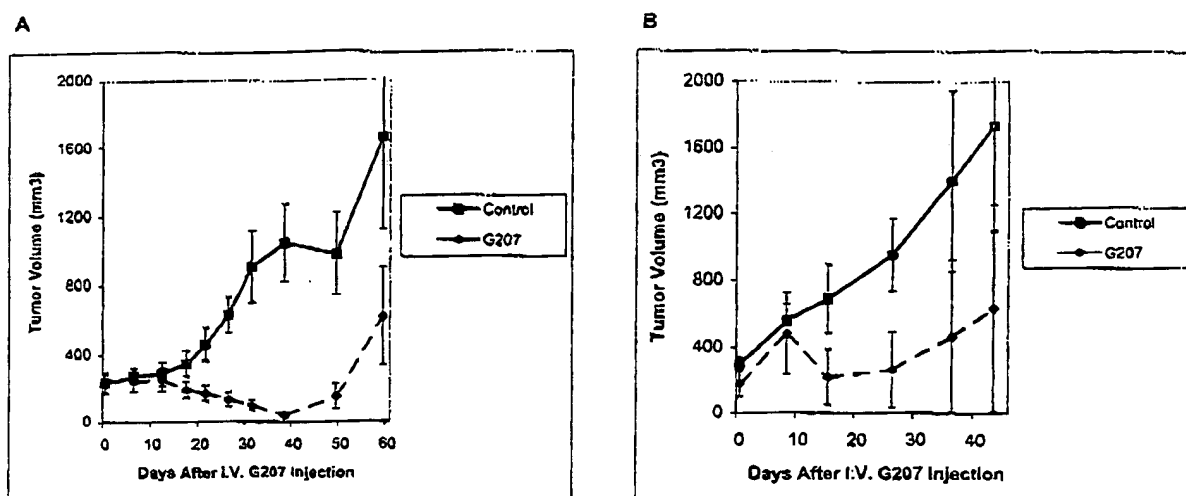


FIG. 4. The effect of intravenous injection of G207 on subcutaneous human prostate tumor growth. (A) LNCaP and (B) DU-145 subcutaneous tumors were established in BALB/c *nu/nu* mice. When the mean volume of LNCaP tumors ($n = 12$) and DU-145 tumors ($n = 12$) was 242 mm^3 (range, $96\text{--}385 \text{ mm}^3$) and 247 mm^3 (range, $63\text{--}510 \text{ mm}^3$), respectively, animals were injected intravenously (via tail vein) on day 0 and day 4 with G207 (2×10^7 PFU) or virus buffer. The mean tumor volume of G207-treated tumors showed a significant reduction versus buffer-treated tumors, which continued to grow (LNCaP, $p < 0.001$ versus controls on day 38; DU-145, $p < 0.05$ versus controls on day 26). Tumor regrowth was noted after the initial reduction in volume of both LNCaP and DU-145 tumors. Complete tumor eradication occurred in 14% (1 of 7) and 40% (2 of 5) of the LNCaP tumors and DU-145 tumors, respectively. (Bars represent mean tumor volume \pm the standard error of the mean.)

two intraneoplastic treatments with G207 caused tumor eradication in 66% of these previously irradiated tumors ($p < 0.05$ versus controls on day 86, *t* test) (Fig. 3). While this total eradication rate is higher than that of Fig. 2, it should be noted that two additional variables must be considered. First, the animals in Fig. 3 received two doses of virus whereas those in Fig. 2 received only one dose. Second, experiments in other tumor systems have shown enhanced antitumor activity when radiation therapy is combined with oncolytic herpes simplex virus (HSV) therapy (Advani *et al.*, 1998). When this is done concomitantly increased viral growth has been documented. An effect seen after temporal spacing could be due to the interactions of apoptotic pathways, injury to tumor vasculature, or other mechanisms worthy of additional study. Whatever the mechanism, it is important to note that prior exposure to radiation did not negatively impact the effectiveness of G207. Thus further preclinical studies are warranted to consider viral oncolytic therapy for patients with recurrent prostate cancer after radiotherapy.

To test the possible use of intravenous delivery of G207 in the treatment of distant metastatic tumors, a preliminary study with subcutaneous DU-145 tumors was performed. DU-145 tumors ($n = 4$) were established subcutaneously in athymic mice and either G207 (2×10^7 PFU) or virus buffer was administered by tail vein inoculation. Animals were sacrificed on day 4, and tumors were removed and stained for β -galactosidase (Mineta *et al.*, 1994). Diffuse areas of LacZ expression were noted in the tumors of G207-treated animals but none was noted in the controls (data not shown). Therefore, LNCaP ($n = 12$) or DU-145 ($n = 12$) tumors were established subcutaneously in athymic mice. These mice were randomized and treated with

either virus buffer or G207 (2×10^7 PFU) by tail vein inoculation. The treatment was repeated 4 days later. G207 caused significant tumor regression or growth inhibition versus virus buffer for both LNCaP ($p < 0.001$ versus controls on day 38, *t* test) and DU-145 ($p < 0.05$ versus controls on day 26, *t* test) (Fig. 4). Although inhibition or regression of tumor growth was observed with the dose regimen used, tumor recurrence was noted in the majority of the animals. Complete tumor eradication occurred overall in one-fourth of the tumors (one of seven LNCaP and two of five DU-145) treated with the two-dose regimen described.

This study has shown that intravenous delivery of G207 can induce tumor growth inhibition, regression, and eradication of distant prostate cancers. Others have demonstrated that localized vascular delivery of replicating HSV vectors is effective in the treatment of experimental brain tumors after intracarotid delivery (Rainov *et al.*, 1998) and in experimental hepatic tumors after hepatic artery delivery (Y. Fong, personal communication, 1999). A word of caution is in order since studies of wild-type and certain attenuated mutant strains of HSV have documented that intravenous administration can produce toxicity of spinal cord, adrenal glands, and liver (Hill *et al.*, 1986; Irie *et al.*, 1998). However, thus far, studies have shown that intravenous delivery of 10^7 PFU of G207 has been nontoxic in BALB/c mice (W. Hunter and P. Sundaresun, personal communication, 1999). Nonetheless, additional preclinical toxicity studies after both intravenous and intraprostatic G207 inoculation are warranted.

The mutations engineered into G207 are important for the significant reduction in neurovirulence after viral infection. G207 has been shown to be nonneurotoxic at 10^7 PFU after di-

rect inoculation into the brain of mice (BALB/c and AJ) (Mineta *et al.*, 1995), the sciatic nerve of mice (G. Mashour, personal communication, 1998), or into the brain of nonhuman primates (*Aotus*) (Mineta *et al.*, 1995). Further, G207 is currently in a phase I clinical trial for the treatment of recurrent malignant glioma and, as of this writing, 3×10^9 PFU has been inoculated intracranially into humans without the production of encephalitis or neural toxicity. Current techniques allow for well-localized stereotactic delivery of a vector to the prostate (Holm *et al.*, 1983). G207 could allow for the *in situ* ablation of prostate cancer cells in a "nerve-sparing" fashion, limiting the incontinence and impotence often associated with radiotherapy or surgery.

In conclusion, G207 may be valuable for treating various stages of prostate cancer including localized, hormonally resistant, previously irradiated, and metastatic tumors. Further improvement of these results may be possible simply by altering the dosing regimen as noted above. In addition, vector modifications providing improved viral replication, or the use of specific promoters, or the expression of cytokines and other anticancer genes, are worthy of development. Preclinical studies of possible toxicity after intravenous and intraprostatic inoculation will be necessary in order to consider this form of therapy for human use.

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Address reprint requests to:

Dr. Robert L. Martuza
Molecular Neurosurgery Laboratory
Georgetown University Medical Center
3800 Reservoir Road N.W.
Washington, D.C. 20007

E-mail: martuzrl@gunet.georgetown.edu

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